Supplemental Materials

A Central Role for LRRK2 in Idiopathic Parkinson Disease

Summary: *Wildtype LRRK2 is activated in dopamine neurons in idiopathic Parkinson disease and plays a pathogenic role in the disease.*

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Materials and Methods

Reagents. Antibodies were sourced as follows:

Antibody	Cat #	Company	Dilution factor
rabbit Anti-LRRK2 (phospho S1292) (MJFR-19-7-8)	AB203181	Abcam	ICC – 1:1000 IHC – 1:500
rabbit Anti-14 – 3 - 3	AB9063	Abcam	ICC - 1:1000 IHC – 1:500
mouse Anti-Synuclein (phospho S129) [P-syn/81A]	AB184674	Abcam	IHC – 1:1000
rabbit Anti-LAMP1	AB24170	Abcam	IHC – 1:500
mouse Anti-p62/SQSTM1	H00008878	Abnova	IHC – 1:500
rabbit Anti-Rab10 (D36C4) Xp(R)	8127S	Cell Signaling	ICC – 1:1000
goat Anti-Rab10 pThr73	S873D	Dr. Dario Alessi	ICC - 1:1000 IHC – 1:500
rabbit Anti-LAMP2A	512200	Invitrogen	IHC – 1:500
mouse Anti-Ndufs3 (OxPhos 30 KDa)	459130	Life Technologies	IHC – 1:1000
sheep Anti-TH	AB1542	Millipore	IHC – 1:2000
rabbit Anti-TOM 20	sc-11415	Santa Cruz	IHC - 1:1000
mouse Anti-LRRK2/Dardarin, clone N241A/34	75-253	UC Davis/ NIH NeuroMab Facility Antibodies incorporated	ICC – 1:1000 IHC – 1:500
rabbit Anti-Iba1	019-19741	Wako	IHC – 1:500

Selective LRRK2 kinase inhibitors were obtained from the following sources: GNE-7915 (ChemieTek), MLi-2 (Dr. Dario Alessi), PF-360 (Pfizer Inc).

Fluorescence measurements. Quantitative fluorescence measurements were made with an Olympus upright 3-laser scanning confocal microscope, taking care to ensure that images contained no saturated pixels. For quantitative comparisons, all imaging parameters (e.g., laser power, exposure, pinhole) were held constant across specimens. Depending on the specific experiment, readouts included fluorescence intensity or number of objects (punctae) in predefined regions of interest, such as tyrosine hydroxylase-positive dopaminergic neurons or Iba1-positive microglia.

Animals. All experiments utilizing animals were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Rats were treated with rotenone as described (*36, 37*).

AAV2-mediated gene transfer. Details per Zharikov et al (*37*). Rats were euthanized 6 weeks after injection.

Human tissue. Paraffin-embedded midbrain sections were obtained from the University of Pittsburgh Brain Bank. All banked specimens have undergone standardized premortem neurological and post-mortem neuropathological assessment. Diagnoses were confirmed and staging performed by the study neuropathologist (JKK) by examination of H&E, alpha-synuclein, tau, silver and ubiquitin stains of key sections needed for Braak staging (*38*). The study design was reviewed and approved by the University of Pittsburgh Committee for Oversight of Research Involving the Dead. Midbrain sections from 7 PD/PDD patients and 8 control subjects, matched for age and postmortem intervals, were used for analysis.

	Male/Female	***Age***	***Brain weight***	***Postmortem Interval***
Control	5/3	67 ± 5	1248 ± 33	7.0 ± 0.8
iPD	6/1	73 ± 4	1297 ± 29	9.8 ± 1.9

To eliminate endogenous fluorescence, human tissue was pre-treated with an autofluorescence eliminating reagent according to the manufacturer's instructions (Chemicon, Temecula, CA).

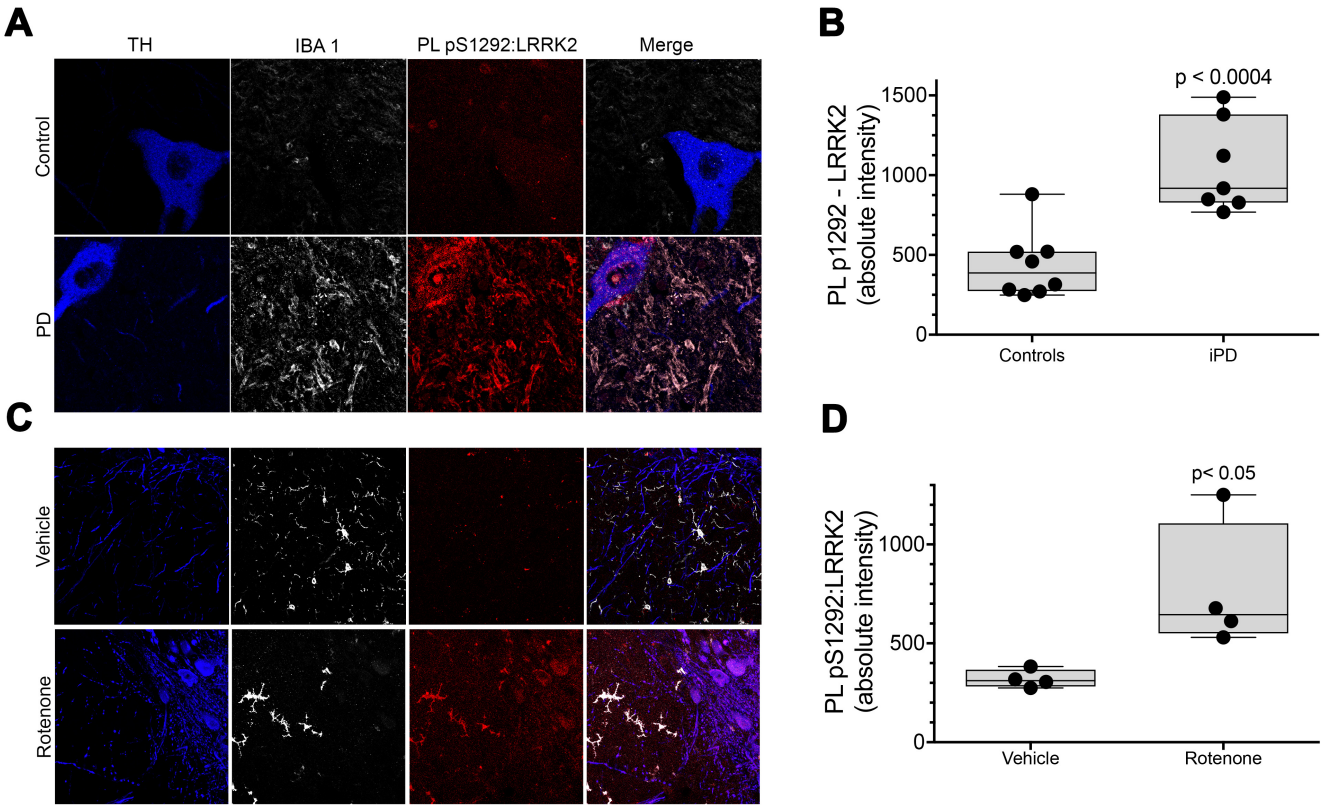

Figure S1. Active LRRK2 is detected by PL in microglia in control brains and is increased in iPD and in rotenone-treated rats. **(A)** Colocalization of pSer1292 PL signal in Iba1 immunoreactive microglia in substantia nigra from control and iPD brains. **(B)** Quantification of pSer1292 PL signal in Iba1 labeled microglia in substantia nigra from 8 control and 7 iPD brains. Comparison by unpaired 2-tail t-test. **(C)** Colocalization of pSer1292 PL signal in Iba1 immunoreactive microglia in substantia nigra from control and rotenone treated rats. **(D)** Quantification of pSer1292 PL signal in Iba1 labeled microglia in substantia nigra from 4 control and 4 rotenone treated rat brains. Comparison by unpaired 2-tail t-test.

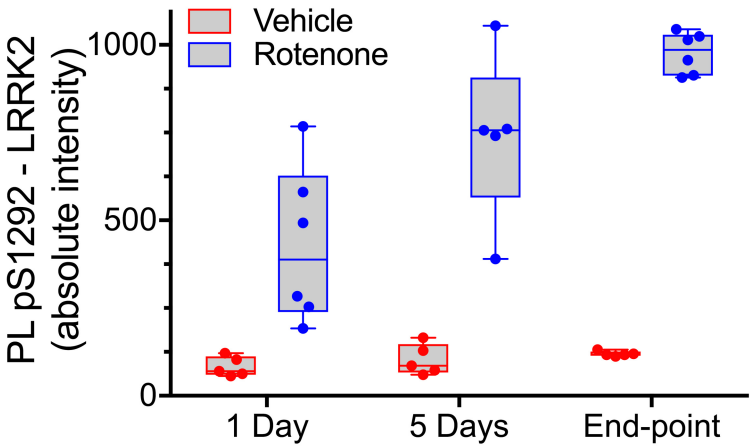

Figure S2. Time course of *in vivo* rotenone-induced LRRK2 activation as assessed by pSer1292 PL signal. Note that LRRK2 is significantly activated by 1 and 5 days of rotenone treatment, time points before there is detectable neurodegeneration. "Endpoint", defined behaviorally, typically occurs at 10-14 days. The pSer1292 PL signal was significantly elevated ($p<0.0001$, ANOVA with Bonferroni correction) compared to vehicle at all time points.

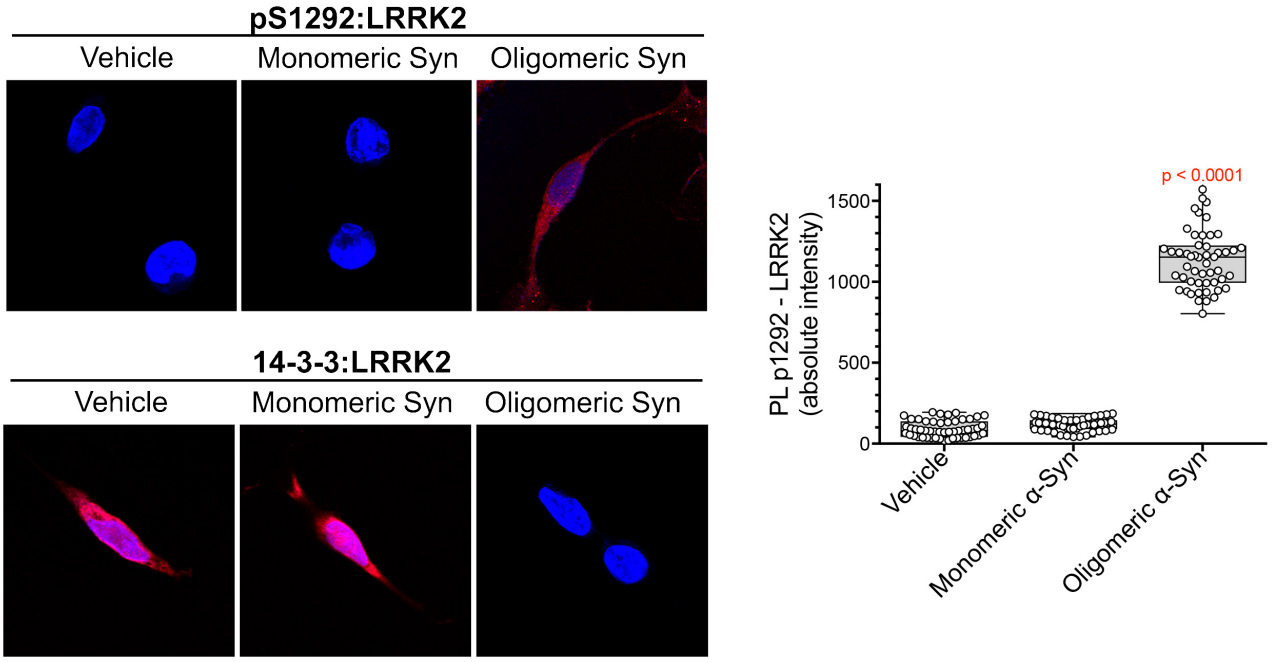

Figure S3. LRRK2 is activated by oligomeric but not monomeric α-synuclein. pSer1292 PL signal (top row) and 14-3-3:LRRK2 PL (bottom row) in SNCA$^{-/-}$ cells treated with vehicle, monomeric α-synuclein or oligomeric α-synuclein. Note the increase in pSer1292 PL signal and loss of 14-3-3:LRRK2 – indicating LRRK2 activation – only in cells treated with oligomeric α-synuclein. The graph shows quantification of the pSer1292 PL signal from these experiments (N=3). Each symbol represents a single cell. Comparison by ANOVA with Bonferroni correction.

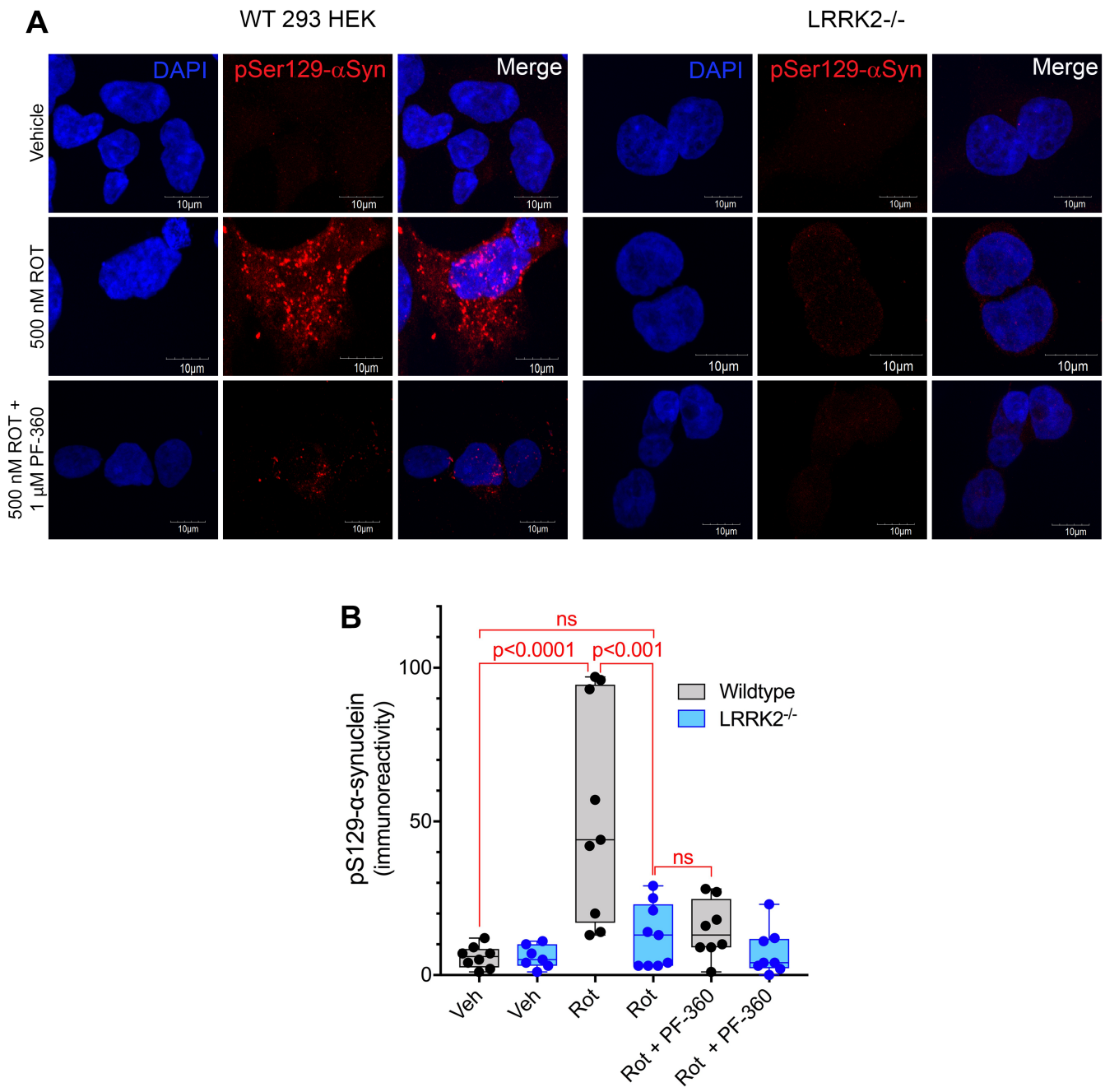

Figure S4. Rotenone-induced accumulation of pSer129-α-synuclein is LRRK2-dependent. Wildtype and LRRK2$^{-/-}$ cells were treated with rotenone with or without co-treatment with 1 µM PF-360. **(A)** Representative images of pSer129-α-synuclein immunofluorescence from wildtype and LRRK2$^{-/-}$ cells. ***Red***, pSer129-α-synuclein immunofluorescence. **(B)** Quantification of pSer129-α-synuclein in wildtype and LRRK2$^{-/-}$ cells 24 hours after treatment with vehicle, rotenone, or rotenone + PF-360. Each symbol represents the mean value of an independent experiment (each with 4 technical replicates) in which immunoreactivity was measured in at least 80 cells. Analysis was by one-way ANOVA with Sidak's multiple comparisons test.